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# Differential regulation of IFN-γ, IL-10 and inducible nitric oxide synthase in human T cells by cyclic AMP-dependent signal transduction pathway

N. BENBERNOU, S. ESNAULT, H. C. K. SHIN, H. FEKKAR & M. GUENOUNOU *Laboratoire d'Immunologie, Faculté de Pharmacie de Reims, France*

## SUMMARY

Expression of cytokines by T lymphocytes is a highly balanced process, involving stimulatory and inhibitory intracellular signalling pathways. In the present work, we attempted to clarify the role of cAMP on interferon-γ (IFN-γ), interleukin (IL)-10, IL-4 and IL-13 expression as well as on the inducible nitric oxide synthase (iNOS) expression. Treatment of phytohaemagglutinin (PHA)/phorbol 12-myristate 13-acetate (PMA)-activated Jurkat cells with either dibutyryl-cyclic adenosine monophosphate (cAMP) or pentoxifylline induced a strong inhibition of IFN-γ mRNA expression as measured by reverse transcription (RT)–polymerase chain reaction (PCR), without affecting IL-10 expression. Both cholera toxin and prostaglandin $E_2$ ($PGE_2$) induced a strong inhibition of IFN-γ mRNA expression, whereas IL-10 mRNA expression was significantly enhanced. This differential regulation of IFN-γ and IL-10 expression was related to intracellular cAMP concentration. IL-13 and IL-4 mRNA expressions were not inhibited. We developed a new method based on immunofluorescence for intracellular cytokine detection followed by optical and computerized image processing, and our results showed that IFN-γ protein was strongly inhibited when cells were treated with $PGE_2$ or dibutyryl (db)-cAMP, whereas IL-10 protein was enhanced. This suggests that cAMP exerts its action at both the transcriptional and protein levels. iNOS mRNA expression was markedly elevated in the presence of $PGE_2$. The generation of nitric oxide using sodium nitroprusside (SNP) induced a dramatic decrease of IFN-γ, while IL-10 was enhanced; and conversely the inhibition of iNOS activity using l-$N^G$-monomethyl arginine (l-NMMA) induced a clear inhibition of IL-10 and IL-4, while IFN-γ was enhanced. These results provide evidence that the protein kinase A (PKA) activation pathway plays a prominent role in the balance between the type 1 and type 2 cytokine profile in PHA/PMA-activated Jurkat cells. Data also suggest that iNOS expression is under the control of PKA activation, and that NO seems to be able to assume the polarization of activated T cells to the type 2 profile.

## INTRODUCTION

Two types of T helper (Th) cells are distinguished by the pattern of cytokine production. Th1 cell clones produce interleukin (IL)-2 and interferon-γ (IFN-γ), whereas Th2 produce IL-4, IL-5 and IL-10. The cytokine profile determines the effector functions of the two subsets of T cells.[1] Th1 and Th2 cell subsets modulate each other's activity, and the balance between the two subsets determines the outcome of infections and pathophysiological diseases. Although not mutually exclusive, Th1 and Th2 responses represent alternate functional modes of the immune system. Several recent reports suggest that upon exposure to antigen, $CD4^+$ cells *in vivo* do shift their cytokine profile in the context of immune response.[2] In particular, in response to allergens and parasites, the Th0 cells appears to lose their ability to express IL-2 while enhancing their ability to produce IL-4.[3,4]

Understanding of the molecular mechanisms that regulate the balance in the expression of Th1 and Th2 type cytokine genes may elucidate fundamental aspects of the immune response.

3′,5′-Cyclic adenosine monophosphate (cAMP) has long been recognized as a second messenger in the control of cellular proliferation.[5,6] Intracellular cAMP levels may be regulated by both adenylate cyclase, which synthesizes cAMP,[7] and cyclic nucleotide phosphodiesterase, which degrades cAMP. cAMP is considered as a potent activator of the protein kinase A (PKA) pathway. It has been demonstrated that cAMP-elevating agents inhibit IL-2 and IFN-γ expression, but

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Abbreviations: db-cAMP, dibutyryl cAMP; CT, cholera toxin; PTX, pentoxifylline; L-NMMA, L-$N^G$-monomethyl arginine; $PGE_2$, prostaglandin $E_2$; M-MLV RT, Moloney murine leukaemia virus reverse transcriptase; SNP, sodium nitroprusside; iNOS, inducible nitric oxide synthase.

Correspondence: Dr N. Benbernou, Laboratoire d'Immunologie, Faculté de pharmacie, Université de Reims, 51, rue Cognacq Jay, 51 100 Reims, France.

not IL-4 and IL-5 expression.[8–10] In this regard, we previously reported a differential cytokine profile in peripheral blood mononuclear cells in response to inhibition of phosphodiesterase by pentoxyfilline.[11] However, little is known about IL-10 and IL-13 expression.

Nitric oxide (NO) is a short-lived radical that has been identified in recent years as a pleiotropic mediator. It is now admitted that NO plays a critical role in the pathophysiology of several diseases.[12,13] NO is synthesized from l-arginine by nitric oxide synthases (NOS), which are expressed either as constitutive (cNOS) or inducible (iNOS) enzymes.[14] iNOS is inducible in murine macrophages by proinflammatory cytokines such as tumour necrosis factor-$\alpha$ (TNF-$\alpha$), IL-1$\beta$, and IFN-$\gamma$. Furthermore, iNOS has been showed to be induced by cAMP in rat vascular smooth muscle cells,[15] while controversial results were described in other cell types such as chondrocytes.[16] There is now increasing evidence that NO may be involved in the signalling between macrophages and T cells,[17] although there is still no evidence that the NO pathway operates in human T cells.

For this study, we asked whether iNOS may be considered as a signalling molecule that is differentially implicated in Th1 and Th2 type cytokine expression following stimulation through the PKA signalling pathway. In the current experiments, we examine and compare the effect of cAMP-elevating agents on IFN-$\gamma$, IL-4, IL-10 and IL-13 as well as on iNOS expression in Jurkat T cells. Our results further substantiate the fact that intracellular cAMP is an important regulator of cytokine profile expression. We also establish a connection between iNOS expression and the differential sensitivity of Th1 and Th2 cells to cAMP.

## MATERIALS AND METHODS

*Cell cultures and reagents*

The human Jurkat T-cell line was maintained in culture in RPMI-1640 supplemented with 10% heat-inactivated fetal calf serum (FCS), 100 $\mu$g/ml streptomycin, 100 U/ml penicillin (Gibco BRL, Life Technologies, Cergy Pontoise, France). Cells were incubated at $1 \times 10^5$ cells/well in 96-well plates, and cultures were stimulated with phytohaemagglutinin (PHA) (10 $\mu$g/ml, Sigma Chemicals, St Quentin Yvelines, France) plus phorbol 12-myristate 13-acetate (PMA) (10 ng/ml, Sigma) for the indicated time points, in the presence or not of prostaglandin $E_2$ ($PGE_2$) (Sigma), dibutyryl-cAMP (db-cAMP, Sigma), cholera toxin (CT, RBI, Illkirch, France) or pentoxifylline (PTX, a generous gift from Hoechst, La défense, France). For certain experiments, cell cultures were performed in the presence of either L-$N^G$-monomethyl arginine (0·5 mM, L-NMMA, RBI) or sodium nitroprusside (1–10 $\mu$M, SNP, Sigma).

*Analysis of mRNA specific for cytokines and iNOS using reverse transcription–polymerase chain reaction (RT–PCR)*

Total RNA was prepared with TRIZOL reagent (Gibco Life Technologies, France). RNA extraction was performed using phenol/chloroform extraction followed by ethanol precipitation. Precipitated RNA (0·4 $\mu$g) from each sample was reverse-transcribed with oligo-dT as the first-strand cDNA primer and Moloney murine leukaemia virus reverse transcriptase $\sigma$M-MLV RT) superscript (Gibco Life Technologies) as previously described.[18,19] Primer sequences for cytokines and for the internal control $\beta$-actin as well as PCR conditions are described elsewhere.[18] Primer sequences for iNOS were as follows: 5′-TCC GAG GCA AAC AGC ACA TTC A-3′ for the 5′ primer, and 5′-GGG TTG GGG GTG TGG TGA TGT-3′ for the 3′ primer. Reaction times for PCR were: 94° 1 min, 66·5° 1 min, and 72° 1 min for 35 cycles.

PCR products were denatured and vacuum dot blotted onto Hybond-$N^+$ membrane (Amersham, Les Ulis, France). Specific probes were 3′-end labelled with fluorescein-11-dUTP using the enhanced chemiluminescence (ECL) 3′-oligolabelling reagents (RPN 2130, Amersham). Probe sequences for cytokines were described elsewhere,[18] and that for iNOS was as follows: 5′-GGG TTG GGG GTG TGG TGA TGT-3′. Following hybridization to the dot blots and incubation with anti-fluorescein-horseradish peroxidase (HRP) conjugate, the detection of the bound peroxidase was peformed using hydrogen peroxide and luminol (RPN 2105, Amersham). The luminescence was detected on blue light-sensitive autoradiography film (Hyperfilm-ECL, RPN 3103, Amersham). The amount of each spot was determined by densitometry analysis (Vilbert Lourmat, Torcy, France). All of the cytokine PCR products were analysed comparatively to the amount of $\beta$-actin detected in the same mRNA sample. Separate cycle course experiments confirmed linearity of amplification for $\beta$-actin, and cytokine cDNA over 20–35 cycles and 30–45 cycles, respectively. For each PCR, linearity of amplification relative to cDNA dilutions was over 1/5–1/20 for IL-4 and IFN-$\gamma$, 1/5–1/40 for IL-13 and 1/5–1/80 for IL-10 and $\beta$-actin.

*Assay of NO synthesis*

After 24 hr of cell incubation, culture supernatants were harvested and the nitrite accumulation was determined by the Griess diazotization reaction (Molecular probe Inc, Eugene, OR,) following a technique previously described.[20]

*Determination of intracellular cAMP concentrations*

Cells were harvested at appropriate time incubation, and collected by centrifugation at 400 ***g***. Cells were resuspended in 65% ethanol (v/v). The extracts were centrifuged at 2000 ***g*** for 15 min at 4°, and dried in a vacuum oven. A commercially available enzyme immunoassay system (RPN 225, Amersham) was used for experiments.

*Immunofluorescence analysis of intracellular cytokines*

Cells were incubated for 48 hr in the presence of PHA/PMA, and for the last 8 hr incubated in the presence of Monensin (2 $\mu$M, Sigma) which blocked the intracellular traffic. Optimal intracellular cytokine staining (for both IFN-$\gamma$ and IL-10) has been achieved using a combination of fixation with paraformaldehyde and permeabilization of cell membranes using Permeafix (OrthoDiagnostics, Roissy, France). Cells were labelled with specific fluoroscein isothiocyanate (FITC)- conjugated monoclonal antibodies (B-T10 for IL-10 and B-B1 for IFN-$\gamma$, Diaclone, Besançon, France). Fluorescence was analysed using image processing. Briefly, conventional images of cells were recorded with a Peltier-cooled CCD camera (C5985, Hamamatsu Photonics, Massy, France) through an inverted microscope equipped with epi-fluorescence (Diaphot 300, Nikon, Champigny-sur-Marne, France). Images were digitalized using an acquisition video interface in $762 \times 570$ pixels

coded in 8 bits, and analysed thereafter in an IBM PC computer using suitable software (Visilog 4, Noesis, Saclay, France). Visilog is a program specializing in image treatment and analysis including image acquisition, filtring and data extraction steps. Fluorescence intensities are mapped in 3D representation using Matlab software (Scientific Software, Sevres, France). Consequently, relative expression of intracellular cytokines in the presence or not of both $PGE_2$ and db-cAMP was quantified.

## RESULTS

### Elevating agents of cAMP downregulate IFN-γ but not IL-10 mRNA expression in Jurkat cells

In order to study whether cytokine gene expression could be modulated by the cAMP-dependent signalling pathway, activated Jurkat T cells were incubated in the presence or not of agents that are known to elevate intracellular cAMP. For all the following experiments, Jurkat cells were stimulated with PHA/PMA.

As shown in Fig. 1., a strong inhibition (93%) of IFN-γ mRNA expression, and a significant enhancement of IL-10 (67%) was noticed in stimulated cell cultures incubated for 24 hr with the analogue of cAMP; dibutyryl-cAMP (1 μM). The same profile of the differential expression of IFN-γ and IL-10 mRNA expression was also observed using cholera toxin (at either the concentrations of 1 or 10 ng/ml).

The involvement of intracellular cAMP in the control of cytokine expression is further confirmed by using pentoxyfilline, which elevates cAMP levels by inhibiting phosphodiesterase and therefore indirectely blocks the degradation of cAMP. As shown in Fig. 1., PTX induced a significant inhibition of IFN-γ mRNA expression relatively to control cells. This effect is dose dependent, and optimal at the concentration of $4 \times 10^{-4}$ M (54% inhibition, Fig. 1a). Using the same concentrations, no inhibition was observed with the expression of IL-10 mRNA (Fig. 1b).

Using $PGE_2$ that is known to physiologically elevate intracellular cAMP levels via receptor-mediated activation of adenylyl cyclase enzymes, we showed that IFN-γ was strongly inhibited in the presence of $PGE_2$ for 24 hr at either the concentrations of 1 and 10 μM (Fig. 1a), while IL-10 mRNA expression was significantly enhanced (Fig. 1b).

No study has yet investigated the sensitivity of IL-13 expression to elevation of cAMP. Our present data showed that the intensity of the signal specific for IL-13 mRNA was not significantly modified in the presence of db-cAMP at either the concentrations of 1 μM and 10 μM (Fig. 1c). A similar profile was obtained with the IL-4 mRNA expression in the presence of the same concentrations of db-cAMP (Fig. 1d).

### Regulatory effects of cholera toxin and PTX on cytokine expression are related to elevation of intracellular cAMP

We have investigated whether the differential expression of IL-10 and IFN-γ was in fact due to elevation of intracellular cAMP. Data showed that cholera toxin induced a large increase of intracellular cAMP (≈164% relative to controls). This effect was observed as from 1 hr of incubation period, reached a maximum at 2 hr (Fig. 2), and decreased afterwards

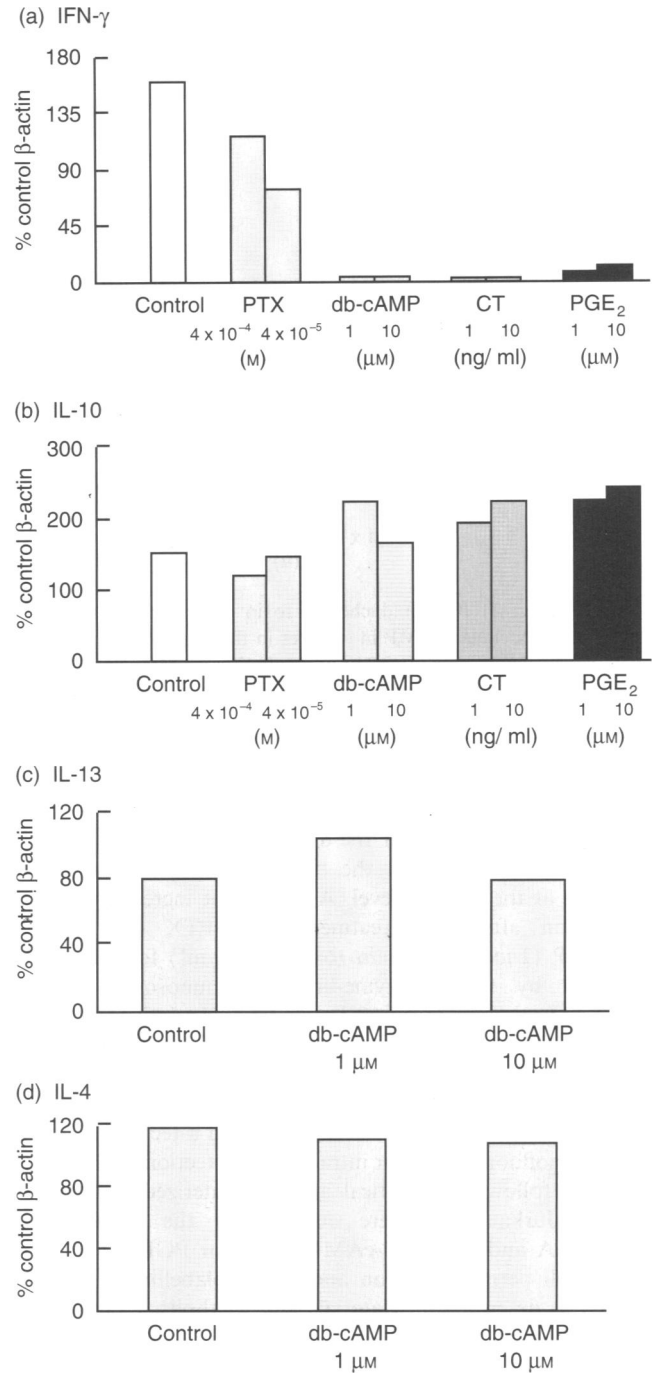


**Figure 1.** Effect of elevating agents of cAMP on cytokines mRNA expression. mRNA expression for IL-10, IFN-γ, IL-13 and IL-4 was analysed in Jurkat T cells. Cells were incubated in culture with PHA/PMA for 24 hr in the presence or not of db-cAMP, PTX, $PGE_2$ or CT. The cytokine PCR products were dot-blotted, hybridized with fluorescein-labelled specific probes, then the ECL system detection was used followed by an autoradiography of the chemiluminescence on a sensitive film. The intensity of the signals was determined by densitometry and normalized to β-actin signal. The relative signal intensities obtained from cell cultures of a representative experiment are presented.

(data not shown). A smaller increase (20% relative to controls) was observed with PTX at $4 \times 10^{-4}$ M after 2 hr of cultures (Fig. 2).

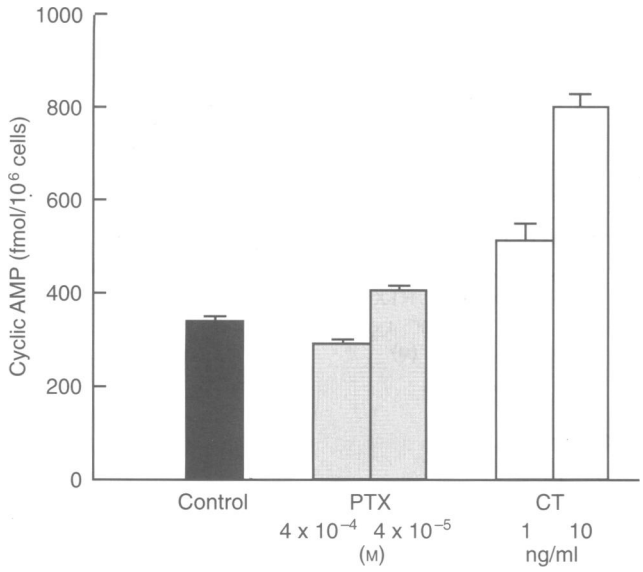


**Figure 2.** Effect of PTX and cholera toxin on intracellular cAMP. Levels of intracellular cAMP in cultures in the presence of either CT or PTX were determined after 2 hr of incubation. cAMP was measured by ELISA in ethanol extracts. Values represent means ±SD of three cultures.

### Analysis of cytokine expression at the protein level

We next analysed whether the differential expression of IFN-γ and IL-10 observed at the transcriptional level was also obtained at the protein level. A significant increase in IL-10 production after cell treatment with PTX (100 μg/ml), db-cAMP (1 μM) or cholera toxin (10 ng/ml) for 24 hr was observed by using enzyme-linked immunosorbent assay (ELISA) (estimated at 2·3-, 3·1- and 3·5-fold relative to control cells, respectively, for PTX, db-cAMP and CT). The measurement of IFN-γ using ELISA did not allow a significant detection even in control cultures (data not shown). In order to improve the cytokine detection, we used a technique based on immunofluorescence for intracellular detection at the single cell level followed by optical and computerized image processing. Jurkat cells were cultured in the presence of PHA/PMA and either db-cAMP (1 μM) or $PGE_2$ (1 μM) for 48 hr. Cell permeabilization and immunolabelling were performed as described in Materials and Methods. Examples of intracellular cytokine detection in Jurkat cells stimulated with PHA/PMA in the presence or not of either db-cAMP or $PGE_2$ are shown in Fig. 3. Fluorescence intensities specific for IFN-γ protein (Fig. 3a) were highly inhibited when cells were treated with $PGE_2$ (Fig. 3b) or db-cAMP (Fig. 3c) relative to control cells (Fig 3a). The magnitude of IFN-γ expression following the effect of either $PGE_2$ or db-cAMP appeared to be significantly correlated from cell-to-cell analysis. In contrast to IFN-γ, IL-10 production was enhanced in the presence of these agents at the same concentrations (Fig. 3b).

### Sensitivity of iNOS expression in Jurkat cells to elevation of intracellular cAMP

When activated *in vitro* with PHA/PMA, Jurkat cells presented significant levels of iNOS mRNA, as measured by RT–PCR. This expression was markedly elevated by the addition of $PGE_2$ at the concentrations of either 1 or 10 μM. Using the same cell preparation, IL-10 mRNA expression was found to be enhanced, whereas IFN-γ mRNA expression was highly inhibited in the presence of $PGE_2$. Results from a representative experiment are shown in Fig. 4 as autoradiographs. Similar results were obtained when we used the db-cAMP analogue (A two-fold increase of iNOS mRNA expression signal in the presence of db-cAMP relative to control cells, as measured by densitometry). The production of NO in culture supernatants could not be detected using the Griess reagents (data not shown).

These results showed that cAMP-induced elevated iNOS expression associated with the elevation of IL-10 mRNA expression and an inhibition of IFN-γ.

We thus investigated the effect of NO directly on the cytokine profile of Jurkat cells in the conditions of PHA/PMA stimulation. Results showed evidence that the generation of NO using SNP (10 μM) induced an inhibition of IFN-γ mRNA expression, while the mRNA expression of IL-10 was enhanced (Fig. 5.). IL-4 mRNA expression was also enhanced in these experiments (data not shown). Higher doses of SNP (100 μM) are damaging for cells under our experimental conditions (data not shown).

Finally, we questioned whether the inhibition of NO synthase influences the cytokine expression. Results showed that in the presence of a potent inhibitor of NO synthase (L-NMMA, at 0·5 mM), IFN-γ mRNA expression was enhanced, while IL-10 as well as IL-4 mRNA expression was inhibited (Fig. 6). This further suggests that the activity of iNOS may be important in the control of Th1 and Th2 cytokines.

## DISCUSSION

Our study presented here clearly demonstrates that the intracellular cAMP is an important regulator of cytokine profile expression upon mitogenic activation. It was already shown that cAMP inhibits IL-2 and IFN-γ expression but not IL-4 production by T cells in human and rodent systems.[8,9,11,21–24] More recently, the combination of cAMP elevating agents and PMA was described to upregulate Th2 promoters such as IL-5 in murine cells.[10] However, no study has yet investigated the sensitivity of IL-10 and IL-13 expression to cAMP signalling pathway in T cells. We have used several strategies to stimulate the cAMP signalling pathway, and data indicate that IL-10 mRNA expression in Jurkat T cells was significantly enhanced upon treatment of cells with cAMP analogue, while IFN-γ mRNA expression was clearly inhibited. In the same experimental conditions, IL-13 mRNA expression was not significantly affected as was IL-4 expression. In the presence of CT, a significant inhibition of IFN-γ mRNA expression was found, whereas IL-10 mRNA expression was enhanced as in the case of the cAMP analogue.

A similar profile of the regulation of IFN-γ and IL-10 mRNA expression was also obtained with the more physiological stimulus of $PGE_2$. These two cytokines are differentially regulated with the same cAMP concentrations. PGEs, which are produced by inflammatory cells such as macrophages,[25] are known to activate membrane adenylate cyclase via receptor–gs-protein coupling, resulting in elevation

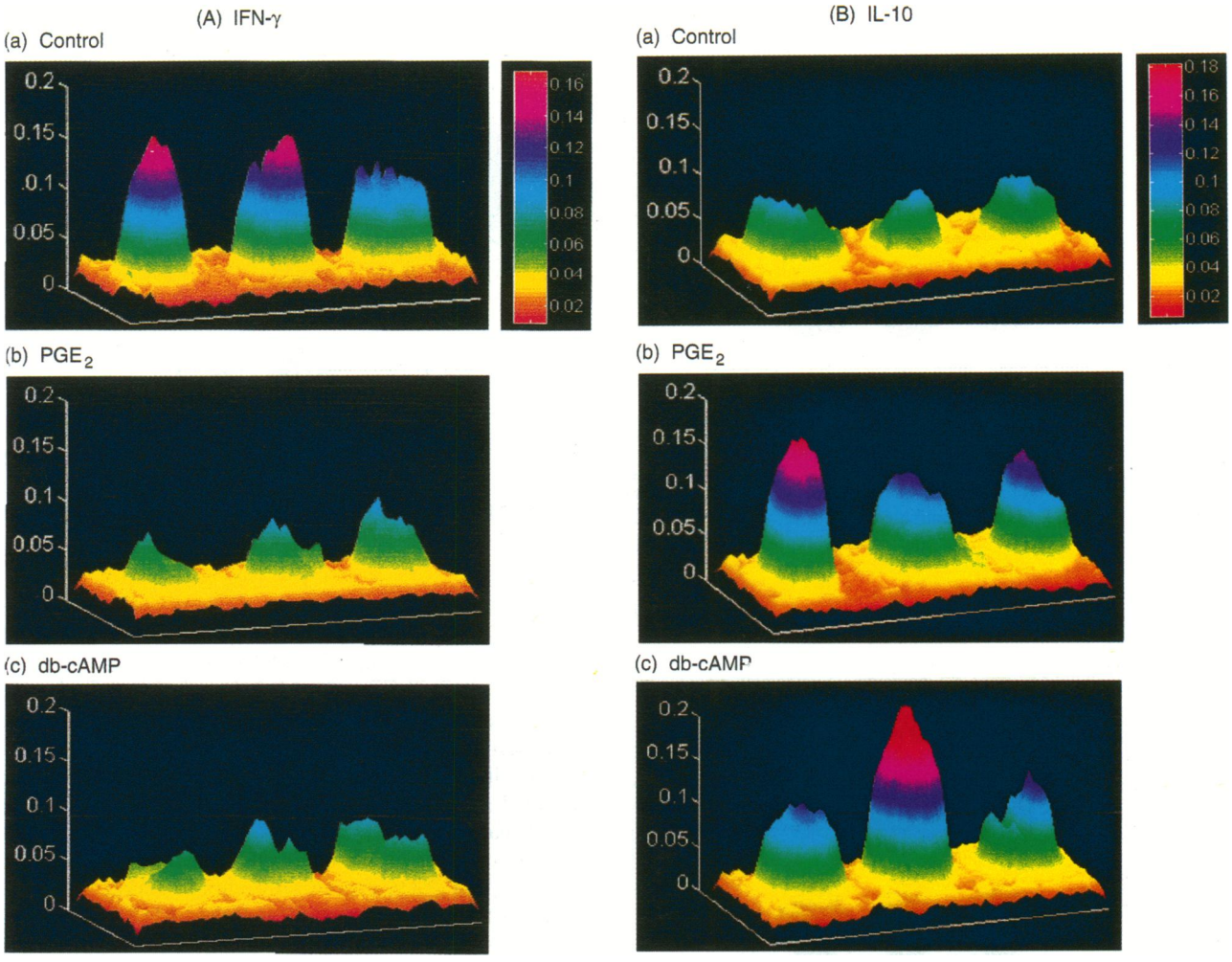


**Figure 3.** Analysis of intracellular cytokines by immunofluorescence and imaging in cell cultures incubated with $PGE_2$ and db-cAMP. Intracellular cytokines were analysed in cells stimulated with PHA/PMA for 48 hr in the absence (control) or in the presence of either $PGE_2$ (1 $\mu$M) or db-cAMP (1 $\mu$M). Cells were permeabilized, and then incubated in the presence of FITC–monoclonal antibody 9mAb) specific for IFN-$\gamma$ (a) or IL-10 (b). Fluorescence images of the cells were acquired and processed, then pseudocolour 3D mapping of fluorescent cells was performed using Matlab software. Intensities were represented as AU (arbitrary units). These intensities are scaled following a h.s.v. (hue saturation value) colour map. The profile of three different cells was presented. Intensities of fluorescence of cells labelled with the irrelevant mAb (mouse IgG1–FITC, Diaclone) presented values that are always below 0·03 AU.

of intracellular cAMP levels. It has already been shown that elevation of cAMP suppresses some functions including cytokine production and antigen presentation.[26] Since the activation of the Th1 cell subpopulation may occur in some inflammatory diseases, one could speculate that the differential sensitivity of Th1 and Th2 cells to $PGE_2$ may represent at least one regulatory mechanism of inflammatory process.

Cytokine regulation was also analysed at the protein level. Using ELISA, IFN-$\gamma$ was not detected, while IL-10 production was found to be significantly enhanced upon treatment with db-cAMP, pentoxifylline or cholera toxin. We developed a new image processing system to measure intracellular cytokine expression using specific fluorescent monoclonal antibodies. This method demonstrated a clear inhibition of IFN-$\gamma$ production in the presence of either db-cAMP or $PGE_2$, while IL-10 production was enhanced in the same culture conditions. Our results strongly suggest that cAMP exerts its action mainly at the transcriptional level.

The influence of protein kinases on the expression of Th1 and Th2 type cytokine profiles remains to be elucidated. Jurkat cells used in this study produced both Th1- and Th2-type cytokines. Our results demonstrated that under activation of PKA pathway, Jurkat cells are switching to a Th2 profile. However, the extrapolation to how Th1 and Th2 cells themselves may behave following PKA activation remains to be elucidated. Indeed, it is not clear whether our results signify that elevation of IL-10 in response to cAMP is a consequence of IFN-$\gamma$ inhibition or that cAMP directly triggers IL-10 synthesis at the trancriptional level. Our results are in accordance with the study of Platzer *et al.* indicating that cAMP elevating agents upregulate IL-10 mRNA expression in monocytes.[27] In addition, it has already been shown that IL-10 is involved *in vivo* in the protective effect of db-cAMP on endotoxin-induced inflammatory injury.[28]

In our experimental conditions, we used a combination of PHA and PMA for stimulation. The same profile of the

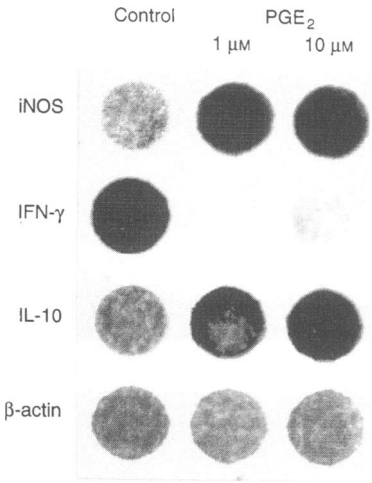


**Figure 4.** PCR analysis of iNOS mRNA expression in Jurkat cells incubated with $PGE_2$. Cells were incubated in culture with PHA/PMA in the presence of $PGE_2$ for 24 hr and mRNA expression of iNOS, IL-10, IFN-$\gamma$ and $\beta$-actin were analysed. The PCR products were dot blotted, hybridized with a specific probe and detected with the ECL system. The results of a representative experiment are presented as autoradiographs.

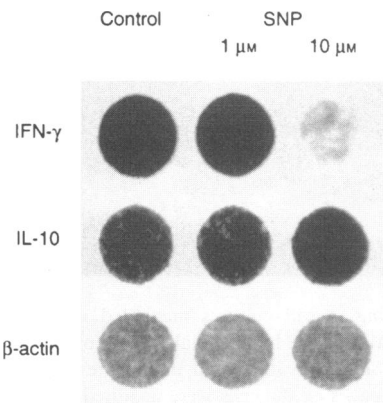


**Figure 5.** Effect of SNP on IFN-$\gamma$ and IL-10 mRNA expression. PHA/PMA stimulated cells, in the presence of SNP, were assayed for mRNA expression of IFN-$\gamma$, IL-10 and $\beta$-actin at 24 hr, as in Fig. 4.

differential control of type 1 and type 2 cytokine expression by cAMP pathway was observed when cells were stimulated with ionomycin and PMA (data not shown). These modes of stimulation mimic T-cell receptor (TCR)–CD3 complex activation, and lead to protein kinase C (PKC) activation and Ca/calcineurin generation that allows NF-$\kappa$B activation.

More recently, tyrosine phosphorylation was also described as being a crucial part of the signal transduction pathway in Th1 cells, as these cells presented a TCR-associated protein tyrosine kinases-phospholipase C-$\gamma$1 (PTKs-PLC-$\gamma$1) transduction, while Th2 cells do not utilize this pathway.[29]

On the basis of our results, we could suggest that elevation of cAMP and consequently, PKA activation could favour a Th2 response in the context of a T-cell response. It is known that allergic patients present an abnormal overexpression of cAMP and $PGE_2$ levels,[30] and increased basal activity of PKA, but decreased basal PKC activity.[31] Furthermore, $PGE_2$ promotes B lymphocyte immunoglobulin isotype switching to

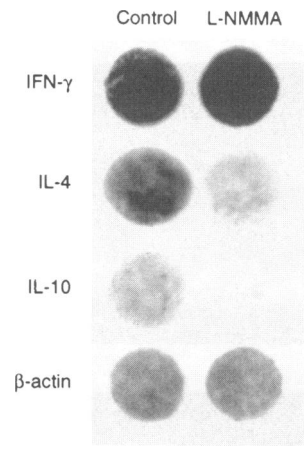


**Figure 6.** Effect of L-NMMA on cytokine mRNA expression. PHA/PMA stimulated cells, in the presence of L-NMMA, were assayed for mRNA expression of IFN-$\gamma$, IL-4, IL-10 and $\beta$-actin at 24 hr, as in Fig. 4.

immunoglobulin E (IgE).[32] So, it seems possible that macrophage production of $PGE_2$ may regulate both T and B cells, stimulating differentiation of Th2 cells as well as promoting IgE production.

NO is an important mediator in the control of physiological processes, and seems to be implicated in the pathogenesis of several diseases.[14] NO is largely produced by cytokine-activated mouse macrophages, fibroblasts, chondrocytes and endothelial cells,[33] although, there is still no evidence that NO pathway operates in human T cells. Our data showed for the first time that iNOS expression occurs in Jurkat T cells, and is influenced by elevation of intracellular cAMP. Indeed, iNOS mRNA expression was clearly enhanced in the presence of $PGE_2$ (Fig. 4). These results are in accordance with other recent reports indicating the induction of iNOS via elevation of cAMP concentration in different types of smooth muscle cells[15] and rat mesangial cells.[34] However, controversial results were recently described in human articular chondrocytes.[16]

The differential expression of iNOS in Jurkat cells may be due to a direct effect of cAMP or it may be a feature of Th2 cells. However, this may be somewhat inconsistent with a previous report of Taylor Robinson indicating that murine Th1 cells, but not Th2 cells, can be activated by antigens or mitogens to produce large amounts of NO.[35] Furthermore, Thüring *et al.* state that iNOS is not present in T-cell clones and T lymphocytes from naive and *Leishmania major*-infected mice.[36] However, in this latter paper, iNOS expression was investigated as an enzymatic activity and at the protein level, and was not analysed by RT–PCR. On the other hand, we could not detect NO production in culture supernatants by using the Griess colorimetric assay (data not shown). So, we suggest that this latter technique may be not sufficiently sensitive to allow detection of a weak NO production as in the case of our experimental conditions.

It may be possible that the increase of iNOS contributes to the inhibition of IFN-$\gamma$ expression in addition to an autocrine activity of the Th2 cytokines such as IL-10, and perhaps, IL-4. This hypothesis is supported by the fact that the generation of NO using SNP in our experimental conditions induced an increase in IL-10 production, which is

associated with a dramatic decrease in IFN-$\gamma$ mRNA expression (Fig. 5). In addition, when we used l-NMMA to inhibit iNOS, the IL-10 expression as well as IL-4 expression was almost completely inhibited, while IFN-$\gamma$ was highly expressed. This is in accordance with the study of Taylor-Robinson *et al.* in murine clones indicating that exogenous NO inhibits IFN-$\gamma$ production.[35] These latter data further substantiate the fact that iNOS expression is not only closely implicated in Th2-type response, but also antagonizes the type 1 response.

It is known that IFN-$\gamma$ acts as a potent co-stimulus for iNOS expression, and that it completely inhibits the IL-10 response.[37] Furthermore, it has already been shown that IL-10 and IL-4 inhibit iNOS in response to IFN-$\gamma$ in macrophages.[38] This signifies that iNOS expression levels may be balanced by positive and negative influences during interaction between macrophages and T cells.

There is evidence that nuclear factors are involved in the differential regulation of cytokine genes. A recent study from Arai and co-workers on the murine cell line EL-4 indicated that NF-AT-related complexes are involved in the differential regulation of IL-2 and IL-5 genes by cAMP.[39] Another study reported the implication of the competition between RelA and NF-ATp on the inhibitory effect of PKC on IL-4 but not IL-2 expression in Jurkat cells.[40] It was also shown that Th2 cells, in contrast to Th1 cells, are lacking in nuclear translocation of NF-$\kappa$B.[41] NF-$\kappa$B was also described as being closely related to iNOS expression,[42] and that NO was found to be able to mediate the regulation of the AP-1 transcriptional factor.[43] The molecular mechanisms of cAMP action on the differential expression of cytokine genes in our experimental conditions and the sequential implication of NO remained to be elucidated.

Taken together, our results provide evidence that PKA activation pathway plays a prominent role in the balance between type 1 and type 2 cytokine profile in activated Jurkat cells. We also shown that iNOS mRNA expression is under the control of PKA activation and is associated with the Th2-type profile, and that NO is also able to polarize activated T cells to Th2-type profile.

## ACKNOWLEDGMENTS

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